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MASTER

PATHOPHYSIOLOGIC EFFECTS OF STABLE IODINE USED AS A THYROIDAL BLOCKING AGENT TO REDUCE THYROID RADIATION EXPOSURE

Final Progress Report

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DISCLAIMER

Introduction

Iodine administration has many undesirable effects on the human thyroid gland. Iodine administration may result in goiter and/or hypothyroidism especially in individuals with autoimmune thyroiditis or those with Graves' Disease previously treated with 131-I or subtotal thyroidectomy. (1,2) Iodine administration may induce hyperthyroidism, especially in areasof iodine deficiency, and hyperthyroidism has also been reported following administration of iodinated radiographic contrast media. (3,4) It has been suggested that the apparent increase in autoimmune thyroiditis in the US population may be related to an increase in iodine intake. (5,6,7,8) Adverse effects of iodine have also been observed in animals. In our laboratory, it has been shown that administration of large doses of stable iodine to iodine deficient dogs causes follicular cell necrosis and discharge of prelabeled organic and inorganic iodine. (9) In hamsters with hyperplastic thyroid glands, administration of iodide produces leukocytic infiltration of the thyroid within 24 hours. This pathologic change is not prevented by administration of glucocorticoids, but resolves after the iodine is discontinued. (10) In guinea pigs, intradermal injection of iodinated lipid may result in chronic thyroiditis. (11)

Increasing amounts of iodine are being administered to the general population in the form of iodinated radiographic contrast media. In addition, iodized oil is being injected intramuscularly in iodine deficient areas as prophylaxis against goiter.(12) Finally, it has been recommended that relatively large doses of iodine be given to populations exposed to nuclear accidents to prevent thyroidal uptake of radioiodine. (13)

In order to determine whether acute administration of iodide leads to any undesireable effects in the general population, we studied a group of patients who received iodinated radiographic contrast medium in the course of routine x-ray diagnostic procedures. We were particularly interested in investigating the possibility that administration of iodine could damage thyroid follicular cells leading to release of intrathyroidal antigens such as thyroglobulin into the blood. An

increase in serum thyroglobulin might, in turn, either initiate or exacerbate thyroid autoimmunity in susceptible individuals, leading to autoimmune thyroiditis. We looked for undesireable effects due to the administration of iodide in 3 ways:

1) A possible acute "toxic" effect on thyroid follicular cells was investigated by determining serum thyroglobulin immediately prior to and 24 hours after injection of iodinated contrast medium.

2) An effect on thyroid autoimmunity was investigated by determining thyroid autoantibodies immediately prior to and 3 - 6 months after injection of iodinated contrast medium.

3) Acute and chronic effects on thyroid function were investigated by performing thyroid function tests immediately prior to, 24 hours after and 3 - 6 months after injection of iodinated contrast medium.

It has not been possible to complete certain aspects of the study as originally proposed. The major problem has been in the question of support funds and time limitations. Each submitted budget has been curtailed and the project further constricted. This has made it particularly difficult to continue the studies on hyperthyroid patients with small pool syndrome with the performance of the iodine kinetics and fluorescence scanning. Some pre-operative patients prepared with propranolol have been given iodine but analysis of their pathologic sections has not as yet been completed. It is hoped that funds for this will be available from other sources since such funds have also been used for other aspects of this study.

The public health aspects of iodine in the project in Nicarauga has finished its first phase. This constitutes extensive control studies on a randomly selected population. Unfortunately, local political problems have delayed the institution of iodination. The second part of the project will follow iodination by 4 to 6 months. The necessary studies of this project are financed from other sources.

MATERIALS AND METHODS

General Outline

After obtaining informed consent, 20 ml blood samples were drawn from patients scheduled to undergo studies which involved administration of iodinated x-ray contrast media. An attempt was made to obtain samples 24 hours after injection of contrast in all patients. Patients were requested to return 3 months or more after the initial study to give followup blood samples. Serum was separated and frozen for later analysis, and all tests on each patient were run in the same assay. Blood samples were analyzed for thyroglobulin (TG), thyroxine (T4), triiodothyronine (T3), thyrotropin (TSH) and for the presence of antibodies to thyroglobulin (TG) and thyroid microsomal antigen (TMA).

Subjects

Any adult inpatient or outpatient scheduled for computerized transaxial tomography with intravenous contrast (CTT), intravenous pyelography (IVP), coronary arteriography or lymphangiography was eligible to be included in the study provided that he or she had not received radiographic contrast media within the previous month. All patients were interviewed by one of us (F.L.) and informed consent was obtained. Eligible patients were included without regard to the presence of thyroid disease or medications. A total of 64 patients were included in the study. Although most had chronic medical problems, only 3 had any history or clinical evidence of thyroid disease. Ages ranged from 24 to 78 (median 55) with 28 females and 36 males.

Radiographic Procedures

40 patients underwent CTT, receiving 120 ml of Conray-60 (meglumine iothalamate 60%) intravenously.

7 patients underwent coronary arteriography, receiving between 115 and 275 ml of Renografin-76 (meglumine diatrizoate 66% and sodium diatrizoate 10%) intra-arterially.

6 patients underwent standard IVP, receiving 50 ml of Renografin-60 (meglumine diatrizoate 52% and sodium diatrizoate 8%) intravenously.

8 patients underwent infusion IVP, receiving 300 ml of Reno-M-Dip (meglumine diatrizoate 30%) intravenously.

3 patients underwent lymphangiography, receiving 10 - 20 ml of Lipiodol (iodinated hydrolized poppy seed oil) intra-lymphatically.

Iodine Content of X-ray Contrast Media

According to the US Pharmacopea, 0.02% by weight of diatrizoate or iothalamate may be present as inorganic iodide in radiographic contrast media. Thus the maximum amount of inorganic iodide which should have been injected was 14 mg for CTT, 30 mg for coronary arteriography, 6 mg for IVP and 18 mg for infusion IVP. Coel et al (14) measured inorganic iodide content of Conray-60 and Renografin 76 and found it to be 4.6 mg/100 ml and 3.7 mg/100 ml respectively. This would result in injection of 5.5 mg of inorganic iodide for CTT and 1.8 mg of iodine for standard IVP. Unfortunately we did not measure the inorganic iodine content of the contrast that was actually injected into our patients. The actual amount of inorganic iodide may vary with length of storage and storage conditions. No data is available regarding the inorganic iodide content of Lipiodol.

Sampling

A blood sample was obtained prior to contrast injection in all patients, and within 24 hours of contrast injection in the majority. Repeat blood samples were obtained 24 hours after injection in 56 patients, and also at 4 or 5 days after injection in 9. 36 patients returned for followup blood samples 2 to 18 months (median 6 months) after injection of contrast.

Analytical Methods

Serum thyroxine and triiodothyronine were determined by standard double-antibody radioimmunoassay using antisera purchased from Endocrine Sciences.

Serum TSH was determined using radioimmunoassay kits purchased from Abbott. Separation of antibody bound and free TSH was by means of polyethylene glycol.

Antibodies to thyroglobulin and thyroid microsomal antigen were determined by the tanned red blood cell hemagglutination technique using kits purchased from Ames (Seratek). The lowest dilution tested was 1:25 and the highest was 1:25,600. Antibodies to thyroglobulin were also determined using a radioassay technique. (See below).

Serum thyroglobulin was determined by a double antibody radioimmunoassay using a modification of the technique of Van Herle et al. (15) Thyroglobulin was isolated from a non toxic goiter and purified by sequential gel chromatography on Sephadex G-200 and Sepharose 4B. The identity and purity of the thyroglobulin was confirmed by sucrose density ultracentrifugation and polyacrilamide gel electrophoresis. The concentration of thyroglobulin was determined by the Lowry technique, using several dilutions performed in duplicate.

4 New Zealand white rabbits were immunized with purifed thyroglobulin emulsified in complete Freund's adjuvent. The initial immunization consisted of 1.0 ml of suspension containing 200 mcg of thyroglobulin injected into the foot pads. One week later two rabbits were given a total of 1.0 ml of a suspension containing 200 mcg of thyroglobulin into the popliteal nodes, while the other two were given 4.0 ml containing 800 mcg of thyroglobulin into the popliteal nodes. All subsequent injections consisted of 200 mcg of thyroglobulin in 1.0 ml of incomplete Freund's adjuvent. Immunizations were made at weekly intervals for an additional two weeks and then at biweekly intervals for 6 weeks. Subsequent boosters have been given at intervals of 3 to 4 months. Serum obtained from the rabbits after the initial 6 weeks of immunization was analyzed by Ouchterlony plate precipitation and immunoelectrophoresis against a variety of antigens. The results demonstrated specificity of the antisera for thyroblobulin, with no cross reactivity to other serum proteins, T4 or T3. In addition, the rabbit antiserum and pooled human serum containing a high titer of anti-thyroglobulin antibodies by the tanned red blood cell hemagglutination technique gave identical precipitation lines when immunoelectrophoresis was performed against purified thyroglobulin. All thyroglobulin assays were performed using a single antiserum obtained 12 months after initial immunization.

Thyroglobulin was labeled with 125-I by a modification of the method of Greenwood (16). The specific activity varied between 50 and 100 mCi/mg. 125-I thyroglobulin was routinely purified by gel chromatography on Sephadex G-200 immediately prior to use in the assay.

Thyroglobulin standards were made up in horse serum, using purified thyroglobulin analyzed by the Lowry method. The buffer used in the assay consisted of phosphate buffered saline (0.01 M phosphate, 0.15 M NaCl) pH 7.5 plus 2% horse serum. Carrier rabbit gamma globulin and goat anti-rabbit globulin were obtained from Antibodies Inc. All standards were run in triplicate and unknown sera in duplicate.

1) 200 ul of patient serum or thyroglobulin standard was incubated with 0.001 ul of rabbit anti-thyroglobulin antiserum (1:900,000) and 60 ng of carrier rabbit gamma globulin in a total volume of 900 ul at 37° C. for 3 hours. (no anti-thyroglobulin was added to the NSB tubes).

2) 0.1 - 0.2 ng of 125-I thyroglobulin contained in 100 ul of buffer was added and the tubes incubated at 37° C. for 17 hours.

3) Sufficient goat anti-rabbit gamma globulin to precipitate all the rabbit gamma globulin in the tubes was added in a volume of 100 ul. The tubes were incubated for an additional hour at 37° C. and then placed in an ice bath for two hours.

4) The tubes were centrifuged at 1,200 X G for 30 minutes in a refrigerated centrifuge at 4° C. The supernatant was aspirated and the precipitates counted in an automatic well scintillation counter for a minimum of 10,000 counts.

5) After subtraction of non-specific binding, bound/total counts were determined for all standards and unknowns. The bound/total for the standards was plotted against the log of the thyroglobulin concentration, and the concentration of thyroglobulin in the unknowns was determined from this standard curve.

125-I thyroglobulin bound in the absence of added thyroglobulin varied between 40 and 60% of the total, and the minimum detectable concentration of thyroglobulin was 2.5 ng/ml. Samples containing more than 160 ng/ml of thyroglobulin were re-run after dilution 1:10 with horse serum. Addition of T4 and T3 in concentrations of 50,000 mcg/ml had no effect on the binding of 125-I Tg to antibody. Serial dilutions of patient sera containing very high levels of thyroglobulin (2,700 and 2,400 ng/ml) in horse serum gave curves which were parallel to the standard curve.

Native antibodies to thyroglobulin which may be present in serum to be assayed for thyroglobulin can interfere with the assay, causing an artifactual increase or decrease in the measured thyroglobulin concentration. The effect depends on the specificity of the anti-rabbit gamma globulin which is used to precipitate antibody bound 125-I thyroglobulin. In either case some of the 125-I thyroglobulin is bound to the native antibodies, which are human gamma globulin. If the precipitating antiserum (anti-rabbit

gamma globulin) is specific and does not cross react with the human gamma globulin, more of the 125-I thyroglobulin will remain in the supernate, leading to a decrease in the precipitated 125-I thyroglobulin and an apparent elevation in the concentration of thyroglobulin in that sample. If the precipitating antiserum cross reacts with and also precipitates human gamma globulin, less of the 125-I thyroglobulin will remain in the supernate and more will be present in the precipitate, leading to an apparent decrease in the concentration of thyroglobulin in that sample. (17) Therefore all sera to be assayed for thyroglobulin were first assayed for antibodies to thyroglobulin using a radioassay technique (vide infra) which is more sensitive than the tanned red blood cell hemagglutination technique. Sera positive for anti-thyroglobulin antibodies were not included in the thyroglobulin portion of the study.

In 23 healthy normal hospital staff members the mean serum thyroglobulin was 8.8 ± 3.2 ng/ml (SEM) and ranged from 4.0 to 16.0 ng/ml.

A radioassay for the presence of antibodies to thyroglobulin was developed using the same materials used in the assay for thyroglobulin. This is a modification of the technique described by Peake. (18)

1) 5 ul of serum was incubated with 0.1 - 0.2 ng of 125-I Thyroglobulin in a total volume of 1.0 ml for 17 hours at 37°C.

2) Sufficient goat anti-human gamma globulin was added to precipitate all the gamma globulin present. The tubes were incubated at 37°C. for an additional hour and then in an ice bath for two hours.

3) The tubes were centrifuged at 1,200 X G for 30 minutes in a refrigerated centrifuge at 4°C. The supernatant was aspirated and the precipitates counted for at least 10,000 counts in an automatic well scintillation counter.

It was found that sera from normal controls with no history or family history of thyroid disease bound less than 6% of the 125-I thyroglobulin. This was assumed to represent non-specific binding. Sera from individuals with detectable antibodies to either thyroglobulin or thyroid microsomal antigen by the tanned red blood cell hemagglutination technique bound over 12% of the 125-I thyroglobulin. Results were expressed as percent 125-I thyroglobulin bound, with values of over 12% considered positive.

The percent 125-I thyroglobulin bound correllated highly with the titer of antibodies to thyroglobulin determined by the tanned red blood cell hemagglutination test. However, the radioassay technique appears to be more sensitive, since it was always positive in patients who had detectable antibodies to thyroid microsomal antigen by the tanned red blood cell hemagglutination test, even when antibodies to thyroglobulin were undetectable by that technique. No individual with a titer of antibodies to thyroglobulin of 1:100 or greater by the tanned red blood cell hemagglutination technique had less than 12% 125-I thyroglobulin bound.

Normal Ranges

Serum thyroxine (T4) - 4.5 to 12.0 mcg/dl

Serum triiodothyronine (T3) - 80 to 200 ng/dl

Serum thyrotropin (TSH) - less than 1.0 to 5.0 microU/ml

Serum thyroglobulin (Tg) - less than 2.5 to 20.0 ng/ml

Antibodies to thyroid microsomal antigen (TMA)-TRC - less than 1:25

Antibodies to thyroglobulin (Tg) - TRC - less than 1:25

Antibodies to thyroblobulin (Tg) - Radioassay - less than 12% 125-I Tg bound

Statistical Analysis

All serum from each patient was run in the same assay. When serum samples were run 10 times in duplicate in the same assay and the results of the 10 pairs of duplicates used to calculate the coefficient of variation, it was less than 2% for thyroxine, triiodothyronine and thyrotropin, and less than 5% for thyroglobulin.

Statistical analysis of the pre and post contrast values for thyroxine, triiodothyronine, thyrotropin and thyroglobulin were made using the Student's test for paired comparisons.

RESULTS

I. Antibodies to Thyroidal Antigens

In 15/64 or 23% of the study population antibodies to thyroglobulin and/or thyroid microsomal antigen were detectable in the control blood sample drawn prior to injection of contrast. 11/28 or 40% of the women and 4/36 or 11% of the men were positive. In 7 individuals (6 women and 1 man) antibodies to both thyroglobulin and thyroid microsomal antigen were detectable. 7 patients had an initial titer of 1:25,600 or greater to at least one antigen. These patients maintained these high titers throughout the period of observation, while of 8 patients who had initial titers of 1:400 or less subsequent samples gave negative results in 50%.

In 11/49 or 22% of the patients who had no detectable antibodies to thyroidal antigens prior to injection of contrast, antibodies were detected in at least one subsequent blood sample. In four instances the elevations were minimal (a tanned red cell titer of 1:25 to only one antigen which was not matched by an increase in the radioassay). However, in seven instances (14% of patients) an increase of at least two tube dilutions and/or a confirmatory increase in the radioassay was observed. In 3 instances (6%) the titers rose from undetectable to 1:25,600 or more. All these individuals were without clinical or laboratory evidence of thyroid disease. To our knowledge none had a positive family history of thyroid disease and none had received x-ray contrast agents previously.

II. Thyroid Function Tests and Thyroglobulin

A. Acute Changes

1. Serum thyroglobulin

As previously noted, serum thyroglobulin could be measured only in those patients who did not have detectable antibodies to thyroglobulin by the radioassay technique. The mean serum thyroglobulin in 39 patients prior to injection of contrast was 10.2 ± 2.3 ng/ml. This is slightly higher than the mean thyroglobulin of 8.8 ± 3.2 ng/ml in a control group of younger hospital personnel. The difference is not statistically significant.

In 26 individuals who underwent CTT scanning mean precontrast serum thyroglobulin concentration was 10.8 ± 1.9 ng/ml. It increased slightly to 11.5 ± 2.0 ng/ml 24 hours later. This increase is not statistically significant ($p > 0.1$). In 6 patients who had thyroglobulin determinations 4 or 5 days after CTT scanning, mean pre-contrast thyroglobulin was 13.6 ± 6.3 ng/ml. This increased slightly to 15.7 ± 5.4 ng/ml. The increase is not statistically significant. In 12 patients who returned for long term followup after CTT, the mean precontrast TG of 13.7 ± 3.0 ng/ml had increased to 15.1 ± 3.0 ng/ml. This increase is statistically significant ($p < .05$).

In 4 patients who underwent coronary angiograpy the pre-contrast thyroglobulin of 6.3 ± 0.7 ng/ml increased slightly to 7.3 ± 1.7 ng/ml 24 hours later. This increase is not statistically significant.

In 10 patients who underwent IVP the mean pre-contrast thyroglobulin of 12.1 ± 3.9 ng/ml decreased slightly to 11.5 ± 3.2 ng/ml 24 hours later. The decrease is not statistically significant.

Serum thyroglobulin could be determined in only one patient who underwent lymphangiography and no change could be detected 24 hours later.

2. Serum triiodothyronine

In 35 patients who underwent CTT scans the mean pre-contrast triiodothyronine was 127 ± 5.0 ng/dl. It decreased to 118 ± 6.0 ng/dl at 24 hours. This decrease is statistically significant ($p < .05$). When patients who were taking medication which could have affected thyroxine metabolism or the concentration of thyroxine binding globulin were eliminated from the analysis, the mean serum triiodothyronine in the remaining 22 patients was 124 ± 6.0 ng/dl. It fell to 113 ± 7.0 ng/dl 24 hours after contrast administration. This difference is also statistically significant ($p < .025$). In one patient triiodothyronine fell from 140 ng/dl prior to administration of contrast to 58 ng/dl 24 hours later. The latter value is well below the lower limit of normal. In the 9 patients who had triiodothyronine determinations performed 4 or 5 days after administration of contrast the control triiodothyronine was 124 ± 10 ng/ml and the post-contrast value 117 ± 10 ng/ml. The difference is not statistically significant ($p > .10$).

In 6 patients who underwent coronary angiography the mean pre-contrast triiodothyronine was 130 ± 7 ng/dl and decreased to 117 ± 9 ng/dl 24 hours later. The decrease is statistically significant ($p < .05$).

In 12 patients who underwent IVP the mean pre-contrast serum triiodothyronine of 109 ± 7 ng/dl decreased to 103 ± 8 ng/dl 24 hours later. This decrease is not statistically significant. However, in one of these patients triiodothyronine fell from 100 ng/dl pre-contrast to 44 ng/dl 24 hours later.

In 3 patients undergoing lymphangiography there was a small increase in serum triiodothyronine from 96 ± 6 ng/dl to 106 ± 5 ng/dl 4 days after contrast administration. This increase is not statistically significant.

3. Serum Thyroxine

In 35 patients who underwent CTT scans the mean pre-contrast serum thyroxine was 7.9 ± 0.3 mcg/dl. It decreased to 7.7 ± 0.3 mcg/dl 24 hours after contrast administration. The difference is not statistically significant ($p > 0.1$). In 9 patients who had thyroxine determinations performed 4 or 5 days after administration of contrast, the mean control thyroxine was 8.1 ± 0.5 mcg/dl and the mean post-contrast serum thyroxine was 7.9 ± 0.4 mcg/dl. The difference is not statistically significant.

In 6 patients who underwent coronary angiography the mean pre-contrast serum thyroxine of 7.8 ± 0.4 mcg/dl increased slightly to 7.9 ± 0.5 mcg/dl following contrast administration. The difference is not statistically significant.

In 12 patients who underwent IVP the mean pre-contrast serum thyroxine of 7.7 ± 0.3 mcg/dl decreased slightly to 7.5 ± 0.3 mcg/dl 24 hours after contrast administration. The difference is not statistically significant.

In 3 patients who underwent lymphangiography the mean pre-contrast serum thyroxine of 5.8 ± 0.7 mcg/dl increased slightly to 6.1 ± 0.5 mcg/dl 4 days later. The difference is not statistically significant.

4. Serum Thyrotropin

In 35 patients who underwent CTT scanning the mean pre-contrast serum thyrotropin of 2.3 ± 0.2 microU/ml was unchanged 24 hours after contrast administration. In 9 patients who had serum thyrotropin determinations performed 4 or 5 days following administration of contrast, the control thyrotropin was 2.0 ± 0.3 microU/ml and the post-contrast thyrotropin concentration was 2.5 ± 0.4 microU/ml. The difference is not statistically significant.

In 6 patients who underwent coronary angiography the mean pre-contrast serum thyrotropin was 2.7 ± 0.3 microU/ml. It increased slightly to 3.0 ± 0.6 microU/ml 24 hours after administration of contrast. The difference is not statistically significant.

In 12 patients who underwent IVP the mean pre-contrast serum thyrotropin of 2.3 ± 0.2 microU/ml was unchanged 24 hours after contrast administration.

B. Late Changes

1. Serum thyroglobulin

In 12 patients who returned for late followup after CTT scanning, the mean serum thyroglobulin was 15.1 ± 3.0 ng/ml versus the pre-contrast mean of 13.7 ± 3.0 ng/ml. The difference is not statistically significant.

In 3 patients who returned for late followup after coronary arteriography, the mean serum thyroglobulin was 5.5 ± 2.6 ng/ml versus a pre-contrast mean of 6.7 ± 0.3 ng/ml. The difference is not statistically significant.

In 6 patients who returned for late followup after IVP, the mean serum thyroglobulin was 5.8 ± 1.1 ng/ml versus a pre-contrast mean of 6.6 ± 1.6 ng/ml. The difference is not statistically significant.

2. Serum tri-iodothyronine

In 21 patients who returned for late followup after CTT scanning, the mean serum tri-iodothyronine was identical to the mean pre-contrast serum tri-iodothyronine of 127 ± 5 ng/dl.

In 2 patients who returned for longterm followup after coronary arteriography, the mean serum triiodothyronine was 113 ± 5 ng/ml versus a pre-contrast mean of 108 ± 3 ng/dl.

In 8 patients who returned for longterm followup after IVP the mean serum triiodothyronine was 128 ± 9 ng/dl versus a mean pre-contrast serum triiodothyronine of 112 ± 8 ng/dl. The difference is not statistically significant ($p > 0.1$).

3. Serum thyroxine

In 21 patients who returned for late followup after

CTT scanning the mean serum thyroxine was 7.5 ± 0.4 mcg/dl versus a mean pre-contrast serum thyroxine of 8.2 ± 0.4 mcg/dl. The decrease is statistically significant ($p < 0.005$).

In 2 patients who returned for longterm followup after coronary angiography, the mean serum thyroxine was 7.1 ± 0.3 mcg/dl versus a mean pre-contrast serum thyroxine of 7.2 ± 0.3 mcg/dl. The difference is not statistically significant.

In 8 patients who returned for longterm followup after IVP, the mean serum thyroxine of 7.7 ± 0.3 mcg/dl was identical to the mean pre-contrast serum thyroxine.

4. Serum thyrotropin

In 21 patients who returned for late followup after CTT scanning, the mean serum thyrotropin was 3.5 ± 0.5 microU/ml versus 2.1 ± 0.2 microU/ml prior to administration of contrast. The difference is statistically significant ($p < 0.01$)

In 3 of the 21 patients the serum TSH had risen above the normal range at longterm followup. In the first, the TSH had increased from 3.2 to 8.0 microU/ml while the serum thyroxine remained constant. In the second, the TSH had increased from 1.2 to 11.5 microU/ml and the serum thyroxine had declined from 8.2 to 5.9 mcg/dl. In the third, TSH had increased from 3.2 to 5.0 microU/ml while serum thyroxine remained constant.

In the 21 patients who returned for longterm followup after CTT scanning, 4 had positive antibodies to thyroidal antigens in pre-contrast blood samples, and 3 had developed antibodies transiently during the period of followup.

In 2 patients who returned for longterm followup after coronary angiography the mean pre-contrast thyrotropin of 1.7 ± 0.2 microU/ml had increased to 2.7 ± 0.3 microU/ml. This increase is not significant.

In 8 patients who returned for longterm followup following IVP the mean pre-contrast serum thyrotropin of 2.4 ± 0.2 microU/ml had increased to 2.8 ± 0.3 microU/ml. This increase is not statistically significant.

Discussion

Thyroglobulin is a glycoprotein with a molecular weight of 670,000. It is the major component of follicular colloid, and is the framework upon which thyroid hormones are synthesized. It was formerly believed that thyroglobulin was a "sequestered antigen" and that autoimmune thyroiditis was caused when thyroglobulin was relaeased into the blood and stimulated a "forbidden clone" of immunologically competent cells to produce antibodies against thyroglobulin. However, it has recently been shown that thyroglobulin is present in the blood of normal individuals in a concentration of about 5 ng/ml. (15) Some thyroglobulin is released into the lymphatics draining the thyroid gland in the course of normal thyroid hormone secretion (19) and reaches the general circulation. The concentration of thyroglobulin is increased in newborn infants, thyrotoxic patients, and following thyroid surgery or treatment of hyperthyroidism with 131-I. It is also elevated in patients with subacute thyroiditis and in some patients with papillary or follicular thyroid carcinoma. (15,20,21)

Although thyroglobulin can no longer be considered a "sequestered" antigen, elevated serum levels of thyroglobulin are often associated with the presence of antibodies to thyroglobulin. In subacute thyroiditis the acute elevation of thyroglobulin is followed by the transient presence of low titers of antibodies to thyroidal antigens (including thyroglobulin) in 50% of patients. (22) Patients treated with 131-I for hyperthyroidism also have acute elevations of thyroglobulin (23) and often either develop antibodies to thyroglobulin or a further elevation in pre-existing thyroglobulin antibodies. (24) In rabbits, injection of their own thyroid tissue combined with adjuvent results in the formation of circulating antibodies to thyroglobulin and in the histologic changes of autoimmune thyroiditis in the remaining thyroid lobe. (25)

Current immunologic theory suggests that tolerance to autoantigens such as thyroglobulin is actively maintained in normal individuals. Three classes of lymphocytes have been distinguished: bursa-equivalent B lymphocytes carry immunoglobulin molecules on their surface. When they come into contact with an antigen

capable of binding to this specific immunoglobulin, they proliferate and produce antibodies against that antigen. Thymus dependent T lymphocytes react to contact with foreign antigens by initiating cellular immune responses. However, they are also responsible for controlling antibody production by B lymphocytes. When a B lymphocyte binds to a foreign antigen the complex is recognized by "helper" T lymphocytes which facilitate antibody production. However, if a B lymphocyte binds to an autoantigen, the complex is recognized by "suppressor" T lymphocytes which prevent the formation of autoantibodies. (26) Killer or K lymphocytes are capable of destroying any cell coated with antigen-antibody complexes. (27) Since normal individuals have lymphocytes capable of binding thyroglobulin in their blood, (28) it is assumed that suppressor T lymphocytes are responsible for the absence of antibodies to thyroglobulin in the majority of normal individuals. (29) This suppression of anti-thyroglobulin antibody production can apparently be overcome in circumstances in which large amounts of thyroglobulin are released into the circulation, such as subacute thyroiditis or following treatment with 131-I. However, in most patients the antibodies to thyroglobulin which are observed under these circumstances disappear after the levels of thyroglobulin return to normal. (22)

It is postulated that autoimmune thyroiditis occurs in individuals who have a genetically transmitted defect in suppressor T lymphocyte function. (29, 30) In such susceptible individuals formation of antibodies might be triggered by release of abnormal amounts of intra-thyroidal antigens. The antibodies produced might, in turn, lead to damage or destruction of thyroid follicular cells. Antigen-antibody complexes have been detected on the basement membranes of thyroid follicular cells by electron microscopy. (31) The presence of such complexes would make the cells susceptible to attack by cytotoxic K cells, which have been recovered from the thyroid glands of patients with autoimmune thyroiditis. (29) Follicular cell damage or destruction would result in further release of intra-thyroidal antigens, perpetuating the process and leading to autoimmune thyroiditis.

The present investigation was designed to determine whether administration of small amounts of iodide in X-ray contrast media leads to release of thyroglobulin and whether this results in the formation of antibodies to thyroidal antigens.

We observed only a small statistically non-significant increase in mean serum thryglobulin levels 24 hours after administration of contrast medium. In a few patients in whom determinations were made 4 to 5 days later, no increase in thyroglobulin was observed. Furthermore, no individual patient had a large increase in thyroglobulin and no patient who subsequently developed antibodies to thyroidal antigen had an increase in thyroglobulin. Despite our failure to document an increase in thyroglobulin in these patients, 11 (22 percent) subsequently developed transiently elevated antibodies to thyroidal antigens.

The transient antibody elevations that we observed appear to be significant, since in most instances the titers of antibodies to thyroglobulin and thyroid microsomal antigen rose and fell in parallel. The separate radioassay method for anti-thyroglobulin antibodies independently confirmed the results obtained with the tanned red blood cell hemagglutination technique. In 3 instances the antibody titers reached levels which would be considered diagnostic for autoimmune thyroiditis.

The significance of transient elevation of antibodies in our hospitalized population is not clear. Low levels of antibodies to thyroidal antigens are found in a significant proportion of normal individuals. In population studies roughly 8% of persons without clinical thyroid disease have detectable antibodies to thyroglobulin, and if antibodies to three thryoidal antigens are tested for, the incidence of positive antibodies rises to 12%. Antibodies are three times more common in women than in men in the younger age groups, but the prevalence rises with age reaching 20% of both sexes after age 70. (32) The post-mortem incidence of focal lymphocytic thyroiditis closely parallels the incidence of detectable antibodies to thyroidal antigens in the general population. Focal lymphocytic thyroiditis is a pathologic process which is microscopically identical to autoimmune thyroiditis of the Hashimoto's type but which occurs as discrete foci surrounded by normal thyroid tissue rather than involving the entire gland. (33)

Detectable antibodies to thyroidal antigens are more common in hospitalized patients than in the general population. In one study using radioassay techniques, 23% of hospitalized patients without clinical thyroid disease had detectable antibodies to thyroglobulin, while 20% had detectable antibodies to thyroid microsomal antigen. (34)

Our data are in good agreement with these. 23% of our hospitalized patients had antibodies to either thyroglobulin or thyroid microsomal antigen or both on the control pre-contrast blood sample. This group included 11/28 women and 4/36 men.

In 7 of our patients with pre-existing antibodies to thyroidal antigens, the titer of antibodies to at least one antigen was 1:25,600 or greater, and did not change significantly during the period of observation. These patients probably had autoimmune thyroiditis despite having normal thyroid glands on palpation. Gordin and Lamberg have followed similar patients and have observed the occurrance of hypothyroidism in 28% over a three year period. (35) In contrast, in those patients who, following injection of contrast medium, developed detectable antibodies to thyroidal antigens during the period of observation, the antibodies disappeared in all patients in whom an adequate followup was obtained. This includes the patients who developed a titer of 1:25,600 or greater, a level usually considered to be diagnostic of autoimmune thyroiditis. The transient nature of the antibody elevations observed in our patients following injection of x-ray contrast medium suggests that they did not develop autoimmune thyroiditis during the period of observation, but that they had a transient loss of tolerance to intrathyroidal antigens.

Other studies have shown that the presence of antibodies to thyroidal antigens may be transient or intermittent. It is well documented that the antibodies which are detected following an episode of subacute thyroiditis virtually always disappear within several months. (22) In a 6 year survey of 5,179 schoolchildren, Rallison et al found that antibodies to thyroglobulin spontaneously disappeared in 1/3 of the children in whom they were present initially. (36) Khangure et al restudied clinically normal individuals who had been found to have antibodies to thyroglobulin during a population survey in 1963. They found that 9 years later only 26% still had positive antibodies, while 12 years later 30% had positive antibodies. (37) The transient presence of detectable antibodies to thyroidal antigens in our patients could have been due either to release of intrathyroidal antigens in an analogy to subacute thyroiditis, or to a transient suppression of T lymphocyte function.

We were unable to document a significant elevation of thyroglobulin in the single measurement made in our patients following injection of iodinated X-ray contrast medium. It is possible that release of thyroglobulin did occur, but that we did not detect it either because the increase in the blood level was too small to be statistically significant or because the elevation occurred between 24 and 96 hours after injection when we did not obtain blood samples. It is also possible that intrathyroidal antigens other than thyroglobulin were released but were not detected because we did not look for them. Alternatively, no increase in the blood level of thryoglobulin or other thyroidal antigens may have occured, either because an insufficient amount of inorganic iodine was injected with the contrast medium or because the population studied was not susceptible to iodine induced follicular cell necrosis. The latter seems the most likely possibility. The dogs which developed follicular cell necrosis following administration of inorganic iodide were severely iodine deficient, presumably with markedly elevated thyrotropin, (9) while our patient population all had normal thyrotropin levels and were presumably iodine replete.

If the transient elevations of antibodies to thyroidal antigens that we observed following injection of contrast medium were not due to an increase in circulating intrathyroidal antigens, they may have been due to transient suppression of T lymphocyte function. Such suppression might permit formation of antibodies to normal levels of intrathyroidal antigens present in the blood. We can only speculate on the possible reasons for suppression ot T lymphocyte function in our patients. It could have been related to injection of contrast medium, but no mechanism for such an effect is apparent. In the absence of a control population this remains a theoretical possibility. It seems more likely that suppression of T lymphocyte function is a characteristic of the population that we have studied. All were hospitalized, many with severe medical problems and most on numerous medications. Previously cited studies have shown an incidence of detectable antibodies to thyroidal antigens in hospitalized populations that is 2 to 3 times that in the normal population. (34) It is possible that the elevations observed in these studies may also have been transient. We are unaware of any other longitudinal studies of the persistance of antibodies to thyroidal antigens following discharge in a hospitalized population.

We observed small decreases in mean serum triiodothyronine concentrations 24 hours after administration of X-ray contrast medium. In patients who underwent CTT or coronary arteriography the decrease was statistically significant, while in patients undergoing IVP it was not. (A small but statistically insignificant decrease in serum T-3 concentration in patients undergoing IVP has been observed by others.)(38) In a few instances a dramatic decrease in serum T-3 occurred resulting in values well below the normal range for the assay. Thyroidal secretion accounts for about 20% of circulating T-3 in normal individuals with the remainder generated by peripheral conversion of T-4 to T-3. (39) The majority of circulating T-3 is bound to serum proteins, primarily thyroid binding globulin, and only about 0.5% is free. The half time of disappearance of T-3 in a euthyroid individual is about 24 hours. (40) The fall in serum T-3 concentrations which we observed could have been due to decreased T-3 secretion by the thyroid, decreased conversion of T-4 to T-3 in the periphery, or displacement of T-3 from protein binding sites resulting in more rapid degradation.

We are unaware of any reported effects of iodinated X-ray contrast media on thyroidal secretion. However, it is well known that iodide will inhibit the thyroidal secretion of T-3 in normal individuals in a dose of 72 mg/day. (41,42) Although we estimate that the total amount of free iodide that was injected into our patients was less than this, in vivo deiodination of as little as 0.2% of the contrast injected for CTT would provide 72 mg of inorganic iodide.

It would seem unlikely that even complete cessation of T-3 secretion by the thyroid would lead to decreases in serum T-3 concentration of the magnitude that we observed in a few of our patients, suggesting an effect on peripheral conversion of T-4 to T-3 and/or T-3 metabolism. There is no evidence that iodide itself has any such effects. (43,44) However, several recent papers strongly suggest that some iodinated X-ray contrast agents do. When sodium ipodate (Oragrafin) is given to hyperthyroid patients or individuals maintained on L-thyroxine, there is a decrease in serum T-3 and an increase in serum reverse T-3. (45) Administration of sodium iopanoate to euthyroid individuals results in a significant decrease in serum T-3 and an increase in reverse T-3, T-4 and TSH seven days later. (38) These observations are consistent

with inhibition of peripheral conversion of T-4 to T-3. The authors of the latter paper propose that this is due to an affinity of the contrast agents for deiodinating enzymes.

We are unaware of any evidence bearing on the possibility that contrast agents might bind weakly to thyroid binding proteins in the blood, displacing T-3 and thus resulting in its more rapid degradation. Another hypothetical possibility without supporting evidence is that the marked hyper-osmolarity induced by large amounts of these agents could have some effect on the peripheral metabolism of T-3 or T-4.

Although there were no acute changes in mean serum T-4 or TSH following injection of contrast, we observed a significant decrease in mean serum T-4 and a significant increase in mean serum TSH 3 to 6 months later. In a few individuals serum TSH was frankly elevated at the time of followup. However, mean serum T-3 was identical to that observed prior to injection of contrast.

A pattern of normal serum T-3, low normal or low serum T-4 and high normal or high serum TSH has been observed in patients with autoimmune (Hashimoto's) thyroiditis and following treatment of hyperthyroidism with radioiodine or thyroidectomy. It is felt to represent early thyroid gland failure or low thyroid reserve. (39,46) In the majority of our patients, serum T-4 and TSH were still well within normal limits. Nevertheless, the pattern does suggest that 3 - 6 months after our initial observation, the thyroid glands in these patients required a greater degree of TSH stimulation to maintain euthyroidism. We can only speculate on the reasons for these observations.

Some of our patients with frankly elevated TSH appear to be developing hypothyroidism. It is possible that the contrast or inorganic iodide damaged the thyroid glands in these patients. There was no correlation between either the thyroglobulin levels following contrast administration or the presence of antibodies to thyroidal antigens (either pre-existing or transient) and the levels of T-4 or TSH 3 - 6 months later. However, the effects may have been too subtle to be detected by the methods used in the study.

It is also possible that the values for T-4 and TSH that we obtained at followup were actually the normal ones for our population, while those obtained prior to injection of contrast had been affected either by the underlying illness or by exposure to the hospital environment. Exposure to small amounts of iodide is inevitable in hospitalized patients. The use of iodine containing skin antiseptics is ubiquitous, and small amounts of iodide are absorbed through normal skin. Low levels of iodine might lead to slight elevation of T-4 with consequent partial suppression of TSH. Both acute and chronic illness and starvation lead to a decrease in peripheral conversion of T-4 to T-3 which may result in a transient increase in serum T-4 and a decrease in serum T-3. (39) In the absence of a control population we are unable to evaluate these possibilities.

1. Vagenakis AG, Braverman LE: Adverse effects of iodides on thyroid function. Med Clin North Am 59: 1075-1088, 1975

2 Wolff J: Iodide goiter and the pharmacologic effects of excess iodide. Am J Med 47: 101-124, 1969

3 Vagenakis AG, Chiu-an W, Burger A, et al: Iodide induced thyrotoxicosis in Boston. N Engl J Med 287: 523-527, 1972

4 Blum M, Weinberg U, Shenkman L, et al: Hyperthyroidism after iodinated contrast medium. N Engl J Med 291: 24-25, 1974

5 Mudge TJ, Sellett T, Nemecek RW: The changing pattern of thyroid disease. Surgery 43: 622-630, 1958

6 Weaver DK, Batsakis JG, Nishiyama RH: Relationship of iodine to lymphocytic goiters. Arch Surg 98: 183-186, 1969

7 Furszyfer J, Kurland LT, Woolner LB, et al: Hashimoto's thyroiditis in Olmsted County Minnesota, 1935 through 1967. Mayo Clin Proc 45: 586-596, 1970

8 Weaver DK, Nishiyama RH, Burton WD, et al: Surgical thyroid disease: a survey before and after iodine prophylaxis. Arch Surg 92: 796-801, 1966

9 Belshaw BE, Becker DV: Necrosis of follicular cells and discharge of thyroidal iodine induced by administering iodide to iodine-deficient dogs. J Clin Endocrinol Metab 36: 466-474, 1973

10 Follis RH: Further observations on thyroiditis and colloid accumulation in hyperplastic thyroid glands of hamsters receiving excess iodine. Lab Invest 13: 1590-1599, 1964

11 Hellwig CA, Wilkinson PN: Experimental production of chronic thyroiditis. Growth 24: 169-177, 1960

12 Buttfield IH, Hetzel BS: Endemic goitre in eastern New Guinea with special reference to the use of iodized oil in prophylaxis and treatment. Bull WHO 36: 243-262, 1967

13 Adams CA, Bonnell JA: Administration of stable iodide as a means of reducing thyroid irradiation resulting from inhalation of radioactive iodine. Health Phys 7: 127-149, 1962

14 Coel N, Talner B, Lang H: Mechanism of radioactive iodine uptake depression following intravenous urography. Br J Radiol 48: 146-147, 1975

15 Van Herle AJ, Uller RP, Matthews NL, et al: Radioimmunoassay for measurement of thyroglobulin in human serum. J Clin Invest 52: 1320-1327, 1973

16 Greenwood FC, Hunter WM, Glover JS: The preparation of 131-I labelled human growth hormone of high specific radioactivity. Biochem J 89: 114-123, 1963

17 Schneider AB, Pervos R: Radioimmunoassay of human thyroglobulin: Effect of antithyroglobulin autoantibodies. J Clin Endocrinol Metab 47: 126-137, 1978

18 Peake RL, Willis DB, Asimakis GK, et al: Radioimmunologic assay for antithyroglobulin antibodies. J Lab Clin Med 84: 907-919 1974

19 Daniel PM, Pratt OE, Roitt IM, et al: The release of thyroglobulin from the thyroid gland into thyroid lymphatics; the identification of thyroglobulin in the thyroid lymph and in the blood of monkeys by physical and immunological methods and its estimation by radioimmunoassay. Immunology 12: 489-504, 1967

20 Torrigiani G, Doniach D, Roitt IM: Serum thyroglobulin levels in healthy subjects and in patients with thyroid disease. J Clin Endocrinol Metab 29: 305-314, 1969

21 Van Herle AJ, Uller RP: Elevated serum thyroglobulin: a marker of metastases in differentiated thyroid carcinomas. J Clin Invest 56: 272-277, 1975

22 Volpé R, Row VV, Ezrin C: Circulating viral and thyroid antibodies in subacute thyroiditis. J Clin Endocrinol Metab 27: 1275-1284, 1967

23 Uller RP, Van Herle AJ: Effect of therapy on serum thyroglobulin levels in patients with Graves' Disease. J Clin Endocrinol Metab 46: 747-755, 1978

24 Hurley JR, Becker DV: Unpublished data

25 Rose NR, Witebsky E: Studies on organ specificity V. changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. J Immunol 76: 417-427, 1956

26 Taylor RB, Basten A: Suppressor cells in humoral immunity and tolerance. Br Med Bull 32: 152-157, 1976

27 Calder EA, Irvine WJ: Cell mediated immunity and immune complexes in thyroid disease. Clin Endocrinol Metab 4: 287-318, 1975

28 Bankhurst AD, Torrigiani G, Allison AC: Lymphocytes binding human thyroglobulin in healthy people and its relevance to tolerance for autoantigens. Lancet 1: 226-230, 1973

29 Allison AC: Self tolerance and autoimmunity in the thyroid. N Engl J Med 295: 821-827, 1976

30 Volpé R, Farid NR, Von Westarp C, et al: The pathogenesis of Graves' Disease and Hashimoto's thyroiditis. Clin Endocrinol (Oxf) 3: 239-261, 1974

31 Kalderon AE, Bogaars HA: Immune complex deposits in Graves' Disease and Hashimoto's thyroiditis. Am J Med 63: 729-734, 1977

32 Doniach D, Roitt IM: Thyroid auto-allergic disease, Clinical Aspects of Immunology. Third edition. Edited by PGH Gell, RRA Coombs, PJ Lachmann. Oxford, Blackwell Scientific Publications, 1975, pp 1355-1386

33 Williams ED, Doniach I: The post mortem incidence of focal thyroiditis. J Pathol 83: 255-264, 1962

34 Mori T, Kriss JP: Measurements by competitive binding radioassay of serum anti-microsomal and anti-thyroglobulin antibodies in Graves' Disease and other thyroid disorders. J Clin Encodrinol Metab 33: 688-698, 1971

35 Cordin A, Lamberg BA: Natural course of symptomless autoimmune thyroiditis. Lancet 2: 1234-1238, 1975

36 Rallison ML, Dobyns BM, Keating FR, et al: Occurrence and natural history of chronic lymphocytic thyroiditis in childhood. J Pediatr 86: 675-682, 1975

37 Khangure MS, Dingle PR, Stephenson J, et al: A long term follow up of patients with autoimmune thyroid disease. Clin Endocrinol (Oxf) 6: 41-48, 1977

38 Bürgi H, Wimpfheimer C, Burger A, et al: Changes of circulating thyroxine, triiodothyronine and reverse triiodothyronine after radiographic contrast agents. J Clin Endocrinol Metab 43: 1203-1210, 1976

39 Schimmel M, Utiger RD: Thyroidal and peripheral production of thyroid hormones: review of recent findings and their clinical implications. Ann Intern Med 87: 760-768, 1977

40 Nicoloff JT, Low JC, Dussault JH, et al: Simultaneous measurement of thyroxine and triiodothyronine peripheral turnover kinetics in man. J Clin Invest 51: 473-483, 1972

41 Mercer CJ, Sharard A, Westerink CJM, et al: Slowing of thyroid secretion by iodide in euthyroid people. Lancet 2: 19-21, 1960

42 Vagenakis AG, Downs P, Braverman LE, et al: Control of thyroid hormone secretion in normal subjects receiving iodides. J Clin Invest 52: 528-532, 1973

43 DeGroot LJ: Action of potassium iodide on thyroxine metabolism. J Clin Endocrinol Metab 26: 778-779, 1966

44 Pittman CS, Shinohara M, Thrasher H, et al: Effect of thyroxine analogues on the peripheral metabolism of thyroxine: the half life and pattern of elimination. Endocrinology 74: 611-616, 1964

45 Wu SY, Chopra IJ, Solomon DH, et al: Changes in circulating iodothyronines in euthyroid and hyperthyroid subjects given ipodate (Oragrafin), an agent for oral cholecystography. J Clin Endocrinol Metab 46: 691-697, 1978

46 Sterling K, Brenner MA, Newman ES, et al: The significance of triiodothyronine (T3) in maintenance of euthyroid status after treatment of hyperthyroidism. J Clin Endocrinol Metab 33: 729-731, 1971